SELECTION OF FOSSILS FOR CALIBRATION OF MOLECULAR DATING MODELS[1]

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Abstract

In the past decade, there has been a rise in interest in the plant fossil record. Fossils potentially provide information for assessing homology and evolutionary change (e.g., the popular missing link phenomenon), character evidence that affects phylogenetic conclusions and, thus, our understanding of modern relationships, evidence of past distributions that can aid in understanding biogeographic histories, and estimates of minimum ages of the clades to which they belong. Recently, many molecular biologists have used fossils in their analyses as a way of providing a calibration point for evolutionary models used to approximate ages for the nodes of phylogenetic trees. However, there has been little, if any, discussion of the criteria by which calibration fossils can be selected for these studies. When considering the use of a fossil as a calibration point, it is critical to take into account the quality of preservation, the method and details of identification (reliability of the taxonomic placement), and the accuracy of the published age. Here, we provide basic criteria for the use of fossils to calibrate molecular evolutionary models. These approaches not only provide better primary estimates for ages of clades, but also provide more reliable sources for those molecular biologists wishing to clean up their molecular clocks.

Key words: Age, calibration point, fossils, geology, identification, molecular clock, preservation, sampling.

Although fossil remains have been the objects of studies since Greek and Roman times, they are frequently ignored by researchers dedicated to molecular and development studies of plants and animals. Nevertheless, knowledge and interpretation of the fossil record are fundamental to understanding the history of life in general, including evolutionary processes. Fortunately, we are witnessing an explosion in the utilization of the fossil record, largely as a consequence of interest in ages of clades, as part of major biogeographical studies, and as a tool for assessing divergence times. Therefore, there has recently been a rise in interest in data obtained from fossils. Although the bulk of the fossil record remains undiscovered, there is no doubt that fossils potentially provide important information on diverse aspects of biology. Fossils are essential for understanding the sequence of evolutionary changes (e.g., the popular missing link phenomenon). They make available character evidence that affects phylogenetic conclusions and assessment of morphologic homology, and, consequently, they influence our understanding of modern relationships among taxa. They also provide support for past distributions that can aid in comprehending biogeographic histories. More importantly, they provide estimates of minimum ages of the clades to which they belong.

During the past 10 years, there has been a tendency to place fossils onto phylogenetic trees (with or without simultaneous cladistic analysis) to calibrate models estimating the age of the obtained nodes or clades (e.g., Bremer, 2000; Bremer et al., 2004; Donoghue et al., 2001; Renner & Meyer, 2001; Renner et al., 2001; Wikström et al., 2001; van Tuinen & Dyke, 2004; Yoo et al., 2005; and others). These methods, such as the nonparametric rate smoothing (NPRS; Sanderson, 1997), semiparametric rate smoothing by penalized likelihood (r8s program; Sanderson, 2002), and Bayesian divergence time estimation (Huelsenbeck & Ronquist, 2001), typically use fossils for calibration. Nevertheless, the fossils often are not included in the analyses performed using these models, and the placement of fossils requires either the results of previous morphological or combined cladistic analyses or acceptance of fossil identifications by paleobotanists. Fossils also have been used extensively for providing historical distributional data in biogeographical studies (Donoghue et al., 2001; Manos & Stanford, 2001; Morley & Dick, 2003; Renner et al., 2001; Wagstaff et al., 2002; Renner, 2004a, b, 2005; and others), but, once more, fossils are usually not included in the primary analyses and, instead, are simply mapped onto molecular phylogenetic trees based on assumptions about their relationships.

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The tendency to map fossils onto phylogenetic trees without including them directly in analyses makes the original identifications and quality of the fossils used extremely important. Although it is refreshing to see that fossils are utilized by molecular biologists to date their trees, it is also disappointing to see that sometimes they do not consider several issues when selecting fossils to be used as calibration points. Most will agree that merely mapping fossils on trees is not optimal because such placements are not directly tested and are, therefore, subjective. More importantly, any conclusion based on the position of phylogenetically untested fossils on a tree will be debatable. For example, Doyle and Donoghue (1993: 151) mapped the "most likely positions of Barremian-Aptian fossils" without directly including them in an analysis onto one of the most parsimonious trees obtained in a previous analysis of only extant taxa. Among the fossils were the earliest (early Cretaceous) putative monocots, represented by a *Liliacidites* Couper pollen grain and *Acaciaephyllum* Fontaine leaf. Later, Gandolfo et al. (2000) actually included the fossils within a cladistic analysis and found that *Acaciaephyllum* and *Liliacidites* were placed in various positions within various clades, but never in the paleoherbs clade in which Doyle and Donoghue placed them with the monocots. Thus, basing a most likely position for a fossil on a generalized identification without cladistic analysis, in this case, has not held up to later scrutiny.

In this paper, we will address various problems associated with fossil selection for calibration of molecular trees and some solutions that should minimize errors in selection of fossils. These recommendations are not meant to replace direct inclusion of fossils in a simultaneous total evidence analysis, which should be undertaken whenever possible, e.g., Hermsen et al. (2003) and Crepet et al. (2005).

THE PROBLEM

For any researcher of paleontology, it is common knowledge that working with fossils can be a tremendous challenge. This challenge has been made more complex by the need to either include fossils directly in modern cladistic analyses or provide strong evidence of their placement in the context of morphological synapomorphies for the groups in question. This often must be accomplished without the help of pre-existing morphological cladistic analyses, resulting in an increasing number of paleobotanical studies in which morphological matrices for modern groups must be constructed in order to place fossils (e.g., Nixon & Crepet, 1993; Crepet & Nixon, 1998a, b; Hermsen et al., 2003). Nixon (1996) reviewed several issues concerning the relationship between traditional paleobotanical work and modern cladistics, including issues of homology, missing data, and reconstruction. Clearly, some of these issues are aggravated when fossils are mapped, without inclusion in an analysis, onto previously calculated phylogenetic trees for calibration of molecular dating models. In a discussion focused on the surprising fossil divergence time between the ancestors of the birds and the mammals of 310 million years ago (Ma) proposed by Hedges et al. (1996) and based on molecular dating, Graur and Martin (2004) listed three sources of error that can influence clade age estimates: (1) errors in the topology of the phylogenetic tree, (2) errors in the taxonomic identification of the fossil material, and (3) errors in the chronological assignment of the geological strata. Combining these concerns about molecular dating per se with concerns about phylogenetic analysis of fossils (Nixon, 1996), we propose that there are several other factors that need to be addressed that impinge on each of the above caveats: (1) fossil preservation (the biased nature of fossil preservation, incompleteness of the fossils or fragmentary taxa, and reconstruction of fossil taxa); (2) methods of identification and taxonomic placement; (3) interpretation of the fossils and establishment of homologies; (4) sampling of taxa/fossils; and (5) the age assigned to the fossils based on the age of the formations where they were collected.

1. FOSSIL PRESERVATION

Biased nature of fossil preservation. Plant fossils can be preserved as compressions, impressions, permineralizations, charcoalifications, and various other less common ways (Taylor & Taylor, 1993; Stewart & Rothwell, 2001). Each type of preservation provides different kinds of data and varying qualities of information. In a permineralization, the fossil usually has preserved internal structure (anatomy) providing details of cell types and tissues. However, some aspects of overall morphology may be difficult to interpret. In compressions, on the other hand, typically only characters of the external morphology and some cuticular features can be observed. Charcoalified (fusainized) fossils are an exception, since they are three-dimensional and mostly show a spectacular array of cell-by-cell preservation, providing a wealth of information unusual in the fossil record (Friis et al., 1986, 1992, 1994, 1995; Friis, 1990; Crepet et al., 1992; Herendeen et al., 1993, 1994, 1999; Nixon & Crepet, 1993; Crepet & Nixon, 1994, 1998a, b; Gandolfo et al., 1998a, b, 2000, 2002, 2004; Hermsen et al., 2003). It is common knowledge that soft parts are more difficult to preserve than hard parts; there are more bones preserved than internal organs of vertebrates and more leaves and fruits than embryo sacs in flowering plants. Based on this, it is reasonable to assume that the majority of fossils are incomplete and, therefore, preservational bias is high. Preservational bias is also dependent on the environment in which fossilization occurs. Fossils are typically preserved better (with less damage and more detail of delicate structures) in environments of low energy (e.g., deltas and lagoons) than in areas of high energy (e.g., high-energy rivers and ash flows).

Incompleteness of the fossils or fragmentary taxa. Although plants typically have many parts that are relatively resistant to immediate decay and tend to preserve well, in general very few complete fossils with attached stems, roots, leaves, and reproductive organs are available in the fossil record. Notable exceptions occur with some aquatics such as *Archaefructus* Sun, Dilcher, Zheng & Zhou, presumably due to in situ preservation in low-energy environments (see Sun et al., 2002). This tendency to fossil fragmentation is further exacerbated by the tendency for some parts to be delicate and/or ephemeral on the living plants. Thus, we may have leaves, fruits, and wood from a single locality, but it is atypical to find a single fossil of the complete organism (attached fruits, leaves, and stems), much less the softer reproductive parts (i.e., flowers). Because of this fragmentary nature of fossils, many characters, even for well-known fossil taxa, are unavailable or incomplete. In the final analysis, most plant fossils are typically represented by single categories of organs (leaves, pollen grains, roots, fragments of stems, reproductive structures, etc.).

Isolated pollen grains, perhaps the most ubiquitous of plant fossils, provide a good example of the difficulties inherent in paleobotanical identification. Common characters studied in pollen grains are size, aperture number and type, and exine sculpture and structure, but determining taxonomic position based on these characters is often very difficult and in general has relied on direct comparisons with living taxa (Zavada, 1983, 1991) rather than comprehensive phylogenetic analyses. Isolated pollen remains difficult to include in large-scale phylogenetic analyses because the available characters that lend themselves to cladistic coding (e.g., aperture number and form) generally conform to very broad groups of taxa, and the details that differentiate smaller clades are often quantitative and labile, or relatively uniform (e.g., fagaceous pollen). Thus, without additional organs, assuming the occurrence of a taxon based on fossil pollen grains alone can be misleading (e.g., *Liliacidites*), such identifications must have less weight than identifications based on a broader range of features. The issue of identifications based on very few dispersed organs (e.g., pollen alone) is very important to address when choosing a fossil as calibration point, in view of the fact that typically only one organ is used for calibration and not the complete plant.

Reconstruction of fossil taxa. Another common practice among paleobotanists is to assemble dispersed parts, usually from the same or nearby localities, into full-organism or partial-organism reconstructions. A well-known case of reconstruction is the extinct genus *Glossopteris* Brongniart, based on an assemblage of variously preserved (but not directly attached) fossil organs including leaves, stems, roots, and a wide array of reproductive organs (e.g., Gould & Delevoryas, 1977). Another interesting case is the corystosperm group of Mesozoic seed ferns. Corystosperms have been considered to have *Rhexoxylon*-type wood based on the association of putatively corystospermous *Dicroidium* Gothan leaves with *Rhexoxylon* Bancroft emend. Archanglesky & Brett stems in the same strata and the presence of similar types of secretory cavities in the two fossils. However, *Dicrodium* leaves now have been found organically attached to fossil stems with *Dadoxylon* Enlitcher–type wood (Meyer-Berthaud et al., 1992, 1993), making the association with *Rhexoxylon* wood ambiguous. These two facts may imply that corystosperms have more than one type of wood; alternatively, perhaps *Rhexoxylon*-type wood is not actually found in the corystosperm clade. The reconstruction problem is even more aggravated for angiosperms. The most common angiosperm organs preserved in the fossil record are leaves and, less commonly, wood, seeds, fruits, and flowers. Unless all the organs are found in organic connection, it is very difficult to produce reconstructions of a whole plant, and there is a tendency to attempt reconstructions based on minimal data. Sometimes, fossils are preserved in the same sediments at the same horizon and they share one or more characters (e.g., the same type of stomata or venation pattern), and, in such cases, a reconstruction seems reasonable. This is the case with *Archaeanthus* Dilcher & Crane, which is based on five fossil taxa independently described by Dilcher and Crane (1984): *Archaeanthus linnenbergeri* (multifollicular fruits), *Kalymmanthus walkeri* (probably bud-scales), *Archaepetala beekeri* and *A. obscura* (putative perianth parts), and *Liriophyllum kansense* (leaves). These organ fossils were found associated but not in organic connection in narrow horizons in different localities, and, furthermore, all these fossils shared the presence of amber-colored resin bodies (Dilcher & Crane, 1984). Nevertheless, without organic connection, reconstructions are always hypotheses to be tested with more data. One approach to testing hypothetical reconstructions is to include the disparate parts in a cladistic analysis to see if, indeed, they can be placed together in the context of parsimony based on the evidence present in each detached organ fossil. This approach was successfully used to test reconstructions of Cretaceous fern fossil organs by Gandolfo et al. (1997).

Another type of fossil reconstruction goes beyond connecting existing dispersed fossil organs and instead utilizes extrapolation of missing parts, usually based on the presence of attachment scars. Such reconstructions are the most highly suspect and, when the identification of the fossil in question relies on such extrapolations, should probably not be used as calibration points for molecular dating. One such extrapolated reconstruction is a putative Nymphaeales/Nymphaeaceae flower based on a single specimen that lacks petals/tepals and stamens. A reconstruction of these organs was presented, indicating filamentous stamens with basifixed anthers and elongate perianth parts based solely on the nature and position of attachment scars around the floral rim (Friis et al., 2001).

2. IDENTIFICATION AND TAXONOMIC PLACEMENT OF FOSSILS

Due to the uncertainty and ambiguity inherent in the fossil record, one of the most challenging aspects of paleontology is the identification and taxonomic placement of fossils. For example, during the late 1800s and until at least the 1980s, remains of leaves, flowers, and fruits of the Tertiary age were mostly described based solely on direct comparisons with extant genera and species based on the premise that for every given fossil there is one extant species that is most similar (Wolfe, 1973). These fossil species were often considered to be direct ancestors, or sister taxa, of extant species without explicit phylogenetic analysis and based on phenetic similarity alone. Unfortunately, these associations were too often based on vague characteristics, and the methodology, known as picture-matching, predominated in paleobotany until the 1980s. The use of this technique produced a great number of fossil misidentifications, as well as a high proportion of incorrect taxonomic placements (for further discussion, see Dilcher, 1973; Hermsen & Gandolfo, 2004).

The most problematic aspect of poor reconstructions, fossil misidentifications, and taxonomic misplacements results from the use of suspect fossil data by non-paleobotanists in studies that utilize fossil occurrences to estimate ages of clades. Such uses are not restricted to molecular dating but also include biogeographical studies in which the age of clades may be used to postulate historical patterns of vicariance and dispersal. Unfortunately, the fossils used in such studies are often not critically evaluated and the identifications and taxonomic affinities are taken for granted from the literature. An example of this problem is provided by Schultheis and Donoghue (2004), who used a fossil assigned to the modern genus *Ribes* L. (*Ribes* species *Axelrod* 1996, UCMP 11156; Fig. 1A) as the oldest reliable fossil occurrence of *Ribes* in North America in a study of the molecular phylogeny and biogeography of *Ribes*. However, a subsequent careful study of the fossil in a review of the group by a paleobotanist revealed a lack of consistent characters with modern *Ribes*, and this calls into question (Hermsen, 2005) any conclusions based on the previous misidentification (Fig. 1A). Another example is a fossil identified as the putatively araceous monocot *Arisaema herperia* Knowlton 1926 (USNM 36885; Fig. 1B). Subsequently, *A. herperia* was used to estimate the age of the Tertiary floristic link between Asia, North America, and East Africa based on a Bayesian chloroplast phylogeny (Renner et al., 2004). The *A. herperia* fossil is based on a single specimen, and Knowlton's identification was performed using the picture-matching method of gross similarity. Hermsen and Gandolfo (2004) reexamined *A. herperia* and found a lack of sufficient characters to make a definitive taxonomic placement with *Arisaema* or the family Araceae. Furthermore, in a previous paper not cited by Renner et al., *A. herperia* had been removed from *Arisaema* and reassigned to *Liquidambar* L. by Brown (1946). Although the taxonomic position of this fossil is still in dispute (Renner et al., 2004; Hermsen & Gandolfo, 2004), it illustrates the difficulties that paleobotanists face in identifying and assigning fossils and the problem of uncritical acceptance of fossil identifications from the older literature. Pollen grains are also frequently used as calibration points (e.g., Bremer, 2000), and these present a challenge as well (see paragraph above).

Different interpretation of the fossils and the establishment of homologies. Nixon (1996) discussed in detail issues of homology assessment in fossils; these will not be repeated here. Different researchers often interpret the same fossil structures differently, sometimes dramatically so, and this increases the uncertainty of homology. Imperfect preservation, lack of context of detached parts, missing features, and lack of precise detail (e.g., anatomy) all contribute to more complex and difficult homology assessment in fossils. Nixon (1996) provided several examples of these issues, including differing interpretations of the homology of the multiovulate cupules of Mesozoic seed ferns such as

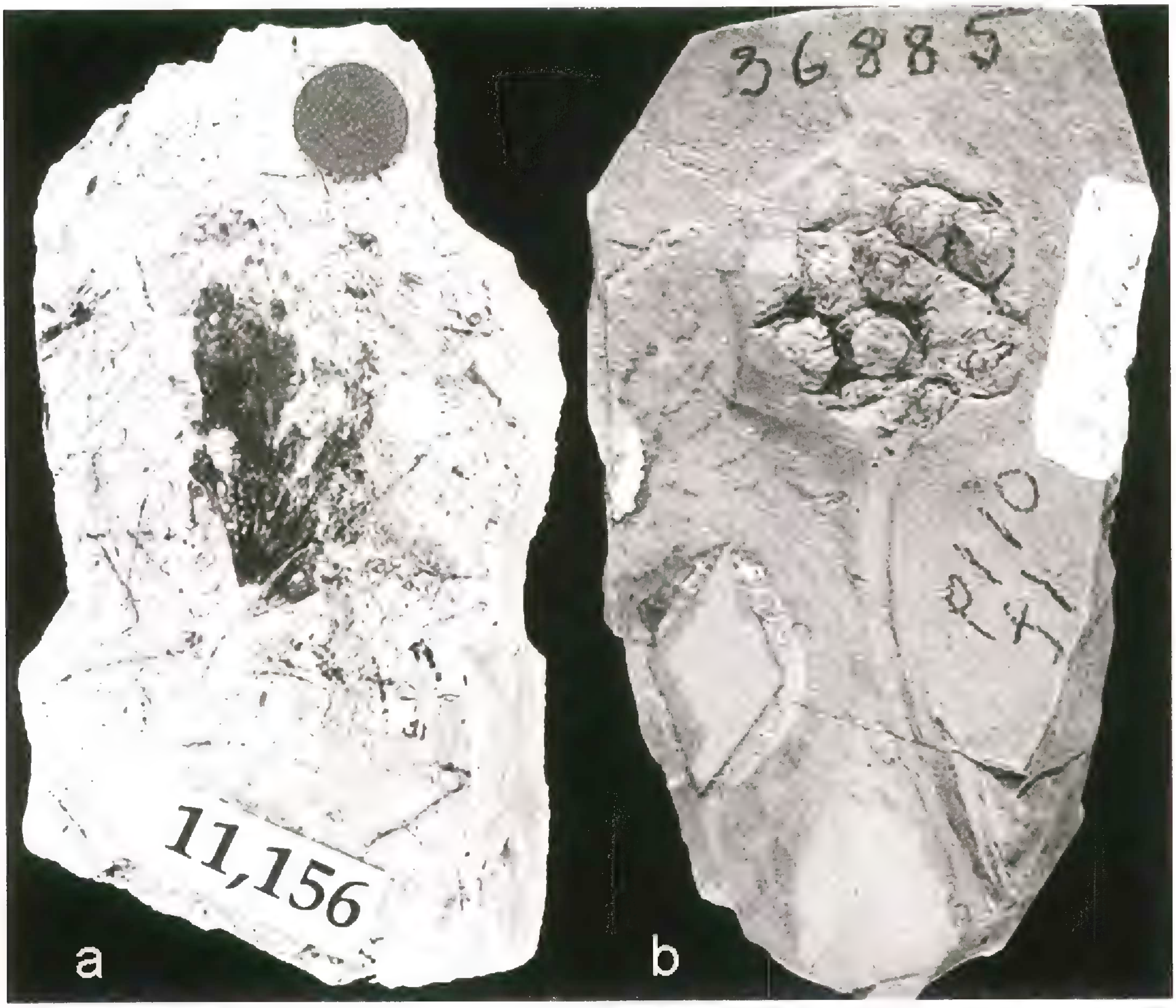

Figure 1. —a. *Ribes* sp. (UCMP 11156) housed at the University of California Museum of Paleontology, Berkeley, California. —b. Type specimen of *Arisaema heperia* Knowlton (USNM 36885) housed at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Caytonia Thomas as compared with angiosperm ovules. Undoubtedly, differences in interpretation may be influenced either by different views of background knowledge (e.g., a given scenario of morphological change) or may be a form of verificationism—fitting our assessment of homology to reflect a given (often popular) view of phylogenetic history. Even when fossils are considered to be very well preserved, there are a limited number of characters one can investigate, and homology assessments are in general weaker than with the extant plants used for comparisons (Nixon, 1996).

3. FOSSIL SAMPLING

Another aspect that has added confusion to the process of the selection of fossils for calibration is the spotty and incomplete fossil record or sample. The incompleteness of the record is apparent in at least three dimensions: temporal, geographic, and taxonomic. Originally, paleontological collections were secondary to particular research programs, and most paleobotany collections were maintained by scientists coming from different backgrounds. The majority were geologists gathering data for physical geological purposes or for commercial endeavors such as mines and oil prospecting. Therefore, the information recorded for specimens within these collections (exact locality, strata and type of environment in which deposition occurred, other fossil associations, etc.) was minimal. As with older herbarium labels, old fossil collections often lack precise location data and other informative facts, and sometimes the information provided on the labels was incorrect. Today, fossil collections are usually made with an emphasis on careful stratigraphic mapping and, more recently, global positioning system waypoints (GPS data) to allow fairly exact relocation of localities and quarries.

Special care is put into documenting vertical and horizontal variations, and, usually, extensive and intensive sampling is undertaken at productive sites. This approach increases the probability of collecting more than a single specimen per taxon, and therefore, taxonomic assignment and identification are more accurate. This more thorough sampling also reduces the proportion of missing data when multiple documented specimens from the same site become available.

4. GEOLOGY AND AGE

Perhaps one of the most pervasive issues related to the utilization of fossils for calibration of molecular dating methods is the tendency to accept the original description, taxonomic assignment, and geologic dating without further investigation (Hermsen & Gandolfo, 2004). As with plant classifications, classification of geological groups, formations, strata, horizons, etc., and their assigned (calculated) age are not static. Thus, when considering a fossil for calibration of a molecular tree, it is necessary to thoroughly search for later modifications of both the taxonomic assignment and, just as importantly, changes in the assigned age. The early angiosperm *Archaefructus* is a good example of an original geologic age that was disputed and subsequently changed from Late Jurassic to Early Cretaceous based on additional data (Sun et al., 2002).

Other issues are purely terminological. Many competing stratigraphic schemes were used until around 1880, when geologists started to address the problem of the necessity of a standard stratigraphic scale. Although it took them more than 100 years to produce the guidelines for defining global chronostratigraphic units, the International Commission on Stratigraphy ([ICS], a commission within the International Union of Geological Sciences [IUGS]) produced the first Global Stratigraphic Chart (Cowie & Basset, 1989), which is revised frequently. Different geologic dating methods often produce different estimated ages for the same deposits. It is now generally accepted that the most accurate ages are obtained by paleomagnetic and radiometric data (U-Th-Pb, Rb-Sr, K-Ar). However, it is perhaps more common to date by other means, such as stratigraphy, biostratigraphy, palynology, and presence or absence of indicator taxa or assemblages of taxa in combination. Both stratigraphic and biostratigraphic (floristic) dating have caveats, including unconformity of strata and the potential for what are considered to be unique floral assemblages to persist or reappear. Therefore, depending on what method has been used to calculate the age of strata, the assigned age and method should be carefully considered when selecting calibration fossils. A clear example of this is the Laguna del Hunco paleoflora that occurs in Patagonia, Argentina. Initially, its age was considered Miocene (23.03–5.33 Ma) based on comparisons with other paleofloras of South America (Berry, 1932), but, based on argon/potassium dating, Archangelsky (1974) suggested a Paleocene age (65.5–55.8 Ma). Mazzoni et al. (1991) later estimated the age at 51–43 Ma based on ignimbrite radiometric dating, and, most recently, Wilf et al. (2003), using paleomagnetic data, determined the age to be early Eocene (52.8–51.7 Ma). Depending on which of these age ranges are used, fossils from this site might produce very disparate results if used for calibration of molecular trees.

The problems of the most accurate date for a particular locality or stratum not only are dependent on dating methods but also are related to the instability of the geological scale, which has changed through time. In 1937, most researchers agreed that the Tertiary started 70 Ma, but today we accept 65.5 Ma as the basal age for this period. Overall, the assignment of the age of the beginning of the Tertiary has fluctuated between 70 and 63 Ma. In addition, the duration of the periods within the Tertiary has changed, with the Oligocene having a 16-million-year duration in 1937 (from 48–32 Ma) to a 17.4-million-year duration in 1982 (from 38–24.6 Ma), and today it has 10.87-million-year duration from 33.9–23.03 Ma (Gradstein et al., 2004; Table 1). Such differences, if not considered when using fossils as calibration points, can also result in widely differing estimates of ages. This kind of variability is observed for all the eras, periods, and stages of the geological scale (Gradstein et al., 2004).

THE SOLUTIONS

It is impossible to completely eliminate problems associated with selection of fossils as calibration points for molecular dating, but such problems as discussed above can be reduced to a minimum if certain steps are taken. This should reduce the errors introduced into such analyses and provide more repeatable results. Undoubtedly, some or perhaps most of the suggested steps that require a paleobotanist or paleobotanical or taxonomic knowledge to undertake may be beyond the scope of many studies. We would hope that the same care and concern should go into the selection of fossils as that which goes into the selection of appropriate genes, methods of phylogenetic analysis, and taxon sampling in the original studies. In many ways, the final result of molecular dating may be more dependent on the selection of a calibration fossil than on the particular

Table 1. Comparison at the period level of selected Paleogene time scales from 1937–2004; modified from Gradstein et al. (2004). Age in million years (Ma). B, beginning of period; E, end of period; D, duration of period.

	Paleogene								
	Paleocene (Ma)			Eocene (Ma)			Oligocene (Ma)		
	B	E	D	B	E	D	B	E	D
1937	70	68	2	68	48	20	48	32	16
1960	70	60	10	60	40	20	40	25	15
1972	65	53.5	11.5	53.5	37.5	16	37.5	22.5	15
1978	65	53.5	11.5	53.5	37	16.5	37	24	13
1982	65	54.9	10.1	54.9	38	16.9	38	24.6	13.4
1985	66.5	57.7	8.8	57.7	36.5	21.2	36.5	23.6	12.9
1987	66.5	54	12.5	54	36	18	36	25.2	10.8
1990	65	56.5	8.5	56.5	35.4	21.1	35.4	23.3	12.1
1995	65	54.5	10.5	54.5	33.7	20.8	33.7	23.8	9.9
2004	65.5	55.8	9.7	55.8	33.9	21.9	33.9	23.3	10.6

gene sequences utilized for the phylogeny, assuming that multiple sequences are selected.

RECOMMENDATION 1: TAXON SELECTION

Verification of the fossil identification:

(a) If possible, verify the identity of the fossil with qualified paleobotanists. Often, suspect fossil taxa may be well known to the paleobotanical community, but there may be relatively little published on these subsequent to the original description. For those who are qualified, it is, of course, better to directly study the actual specimen(s) if possible.

(b) Evaluate the original diagnosis provided by the author of the fossil genus/species. In many older papers, fossils lack a formal diagnosis, and such fossils are immediately suspect in terms of their identity. At a minimum, some characteristics that are unique to the group to which the fossil is assigned should be determined. If it is not possible to characterize the fossil with a diagnosis that clearly associates it with its presumed modern relatives and eliminates assigning it to multiple other taxa, it should probably be excluded immediately as a candidate for a calibration point.

(c) Check for all taxonomic/nomenclatural changes subsequent to the original publication. Sometimes, this is sufficient to eliminate a fossil from consideration.

(d) Check for synapomorphies that provide unequivocal taxonomic assignment of the fossil. If the fossil lacks synapomorphies or at least an assemblage of characters that confirmed its taxonomic placement, it should be rejected and not used as a calibration point.

(e) If the fossil taxon has not been previously included in a phylogenetic analysis, it should be, if at all possible. This can often be done by including the fossil taxon in an existing morphological analysis of modern taxa that are within the scope of the identification.

RECOMMENDATION 2: FOSSIL SOURCE

(a) Confirm the data associated with the collection of the fossils: formation, strata, and locality. Initially, these should be obtained from the original paper, but because concepts of formation and strata can change, these need to be verified.

(b) Verify that the specimen(s) are still available. This may require contacting the presumed collection in which the specimen is housed. Any fossil taxon used as a calibration point should have available specimens and, presumably, a clearly designated type specimen, so that future studies can evaluate the quality of the assignment.

RECOMMENDATION 3: AGE

(a) Confirm the age of the associated formation, strata, and locality. Again, because concepts change and strata are sometimes remapped, it is important to determine if any changes have occurred since publication of the fossil.

(b) Check in the geological literature for changes in the estimated age of the strata since the date of original publication of the fossil.

(c) Use the youngest, most probable, most precise period (if the period is Eocene, use 33.9 Ma and not 55.8 Ma).

(d) Provide the correct bibliographic citation for the age adopted and, in particular, the source of any revised age estimates for the strata in which the fossil(s) occur.

CONCLUSIONS

The use of fossils as calibration points in molecular dating is an exciting and potentially useful endeavor, independent of which molecular dating methods are used or the theoretical justifications for particular approaches. The renewed focus on fossils in this context has exposed numerous issues that have been well known to paleobotanists, but may not be apparent to workers seeking to simply obtain a calibration point as easily as possible. Providing carefully identified and accurately aged fossils that can be utilized by molecular biologists in such studies is the responsibility of the paleobotanist. Unfortunately, the vast body of paleobotanical work is largely inappropriate for use to calibrate molecular trees without careful scrutiny and attention to issues related to traditional, often nonrepeatable, approaches to fossil study. The paleobotanist must be a primary partner in these endeavors, and, hopefully, paleobotanists will increasingly become collaborators in studies that use molecular dating methods. At a minimum, molecular botanists should carefully consider which fossils they are using, and paleobotanists should seek to provide fossils in a verifiable phylogenetic context based on combined molecular and morphological analyses. In these ways, both fields of endeavor will be improved.

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